DUPLICATE

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PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

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Applicant's or agent's file reference 609225C:GDR	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International Application No. PCT/AU2003/000249	International Filing Date *(day/month/year)* 28 February 2003	Priority Date *(day/month/year)* 8 October 2002

International Patent Classification (IPC) or national classification and IPC

Int. Cl. ⁷ C07K 14/47, 16/18; C12N 5/20, 9/64

Applicant

UNISEARCH LIMITED et al

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets, including this cover sheet.

[X] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 3 sheet(s).

3. This report contains indications relating to the following items:

- I [X] Basis of the report
- II [ ] Priority
- III [ ] Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV [ ] Lack of unity of invention
- V [X] Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI [ ] Certain documents cited
- VII [ ] Certain defects in the international application
- VIII [ ] Certain observations on the international application

Date of submission of the demand	Date of completion of the report
5 May 2004	13 September 2004
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer **CHRISTINE BREMERS** Telephone No. (02) 6283 2313

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/AU2003/000249

I. Basis of the report

1. With regard to the elements of the international application:*

- [ ] the international application as originally filed.
- [X] the description, pages **1, 3-4, 6-31 and abstract page 36**, as originally filed,
  pages , filed with the demand,
  pages **2 and 5**, received on **18 August 2004** with the letter of **17 August 2004**
- [X] the claims, pages **32-35**, as originally filed,
  pages , as amended (together with any statement) under Article 19,
  pages , filed with the demand,
  pages , received on with the letter of
- [ ] the drawings, pages **1/8-3/8 and 5/8-8/8,** as originally filed,
  pages , filed with the demand,
  pages **4/8,** received on **18 August 2004** with the letter of **17 August 2004**
- [X] the sequence listing part of the description:
  pages **1/5-5/5**, as originally filed
  pages , filed with the demand
  pages , received on with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

- [ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).
- [ ] the language of publication of the international application (under Rule 48.3(b)).
- [ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- [ ] contained in the international application in written form.
- [X] filed together with the international application in computer readable form.
- [ ] furnished subsequently to this Authority in written form.
- [ ] furnished subsequently to this Authority in computer readable form.
- [ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- [ ] The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. [ ] The amendments have resulted in the cancellation of:
   - [ ] the description, pages
   - [ ] the claims, Nos.
   - [ ] the drawings, sheets/fig.

5. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

V. **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-33	YES
	Claims		NO
Inventive step (IS)	Claims	1-33	YES
	Claims		NO
Industrial applicability (IA)	Claims	1-33	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

Novelty and Inventive Step

D1 Database GenBank Accession Number AAK61272, submitted 7 December 2000
& DANIELS R J et al, "Sequence, structure and pathology of the fully annotated terminal 2 Mb of the short arm of human chromosome 16". Hum. Mol. Genet. 2001 Vol 10 no 4 pages 339-352

D2 PALLAORO, M et al "Characterization of genes encoding known and novel human mast cell tryptases on chromosome 16p13.3". J. Biol. Chem. 1999 vol 274 no 6 pages 3355-3362.
& Database GenBank Accession Number AAD17845, submitted 8 October 1998
& Database Swiss Prot Accession Number Q9BZJ3, submitted ~November 1999
& Database RefSeq Accession Number NP 036349 provisional sequence

D3 MIN, H K et al, "Human mouse mast cell protease 7-like tryptase genes are pseudogenes". J. Allergy Clin. Immunol. 2001, vol 107 no 2 pages 315-321.
& Database GenBank Accession Number AAK12909, submitted 1 November 2000
& Database Swiss Prot Accession Number Q9BZJ3, submitted ~November 1999
& Database RefSeq Accession Number NP 036349 provisional sequence

D4 WANG, H-W et al, "δ Tryptase is expressed in multiple human tissues, and a recombinant form has proteolytic activity". J. Immunol. 1 November 2002, vol 169 no 9 pages 5145-5152
& Database Swiss Prot Accession Number Q9BZJ3, submitted ~November 1999
& Database RefSeq Accession Number NP 036349 provisional sequence.

These documents were cited in the ISR.

D4 Wang et al (the closest prior art as shown in Fig 3 of Wang) was published after the priority date of the present application. As the claimed priority date appears to be valid, D4 Wang et al is not relevant prior art according to R.64.1PCT.

D1-D3 disclose the predicted amino acid sequence of a δ tryptase based on the nucleotide sequence of the gene. There is no evidence of expression of the gene. D3 in particular indicates that δ tryptase genes are pseudogenes and hence have no function and are not expressed.

Therefore claims 1-33 are considered novel and inventive in light of D1-D3.

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Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of Box V

Industrial Applicability

Claims 1-33 are directed to tryptase polypeptides useful in the diagnosis of inflammatory disease and therefore satisfy the criteria for industrial applicability.

functional human tryptases are expressed. A number of tryptase genes are known to be grouped on chromosome 16, mapping to 16p13.3. These include the gene encoding βI tryptase and its allelic partner αII tryptase, the allelic genes encoding βII and βIII tryptase, two allelic variants of a transmembrane tryptase called gamma (γ) tryptase, and two allelic variants of another tryptase originally named "mMCP-7-like" (Pallaoro *et al.*, 1999; Caughey *et al.*, 2000). Of these, cDNAs have been cloned for all loci except for that encoding "mMCP-7-like" tryptase. Recently the cloning of a more distantly related member, epsilon (ε) tryptase, which is approximately 40% similar to the α/β tryptases has also been described (Wong *et al.*, 2001).

The mMCP-7-like tryptase was so named due to homology between its fifth exon and the murine tryptase mouse mast cell protease (mMCP)-7 (Pallaoro et al., 1999; McNeil et al., 1992). Recently it has been reported (Min *et al.*, 2001) that the mMCP-7-like gene is not transcribed. Based on the examination of a number of human tissues and cell lines for transcription of the mMCP-7-like gene, Min *et al.* reported that mRNA is absent and concluded that the mMCP-7-like gene is a pseudogene. However the present inventors have surprisingly discovered that the human mMCP-7-like tryptase is indeed expressed and have named the gene, and its polypeptide product delta (δ) tryptase.

Summary of the Invention

According to a first embodiment of the present invention there is provided a purified, expressed δ tryptase polypeptide or fragment or analogue thereof. Preferably, the expressed δ tryptase polypeptide is the human δ tryptase protein. More preferably, the expressed human δ tryptase protein has the amino acid sequence as set forth in SEQ ID NO:1 or SEQ ID NO:2, or the amino acid sequence as set forth in SEQ ID NO:1 or SEQ ID NO:2 including one or more conservative amino acid substitutions.

According to a second embodiment of the present invention there is provided a purified, expressed variant of the δ tryptase polypeptide of the first embodiment, or a fragment or analogue of this variant. Preferably, the variant δ tryptase polypeptide is the product of alternative splicing of the primary RNA transcript. More preferably the variant polypeptide has the amino acid sequence as set forth in SEQ ID NO:3.

In a third embodiment the present invention provides a recombinant host cell expressing the polypeptide or fragment or analogue thereof of the first or second embodiment.

In a fourth embodiment the present invention provides an antibody that selectively binds to the polypeptide or fragment or analogue thereof of the first or second embodiment.

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Alternatively, or in addition, the kit may contain an antibody of the fourth embodiment. Preferably the kit is used for carrying out the methods of the fifth, to the tenth embodiments.

Brief Description of the Drawings

Preferred embodiments of the present invention will now be described, by way of example only, with reference to the accompanying drawings.

Fig. 1. RT-PCR amplification of the transcript for δ tryptase. **A)**. RT-PCR of total RNA isolated from the HMC-1 cell line using two primer pair combinations, F1/R1 and F2/R2. GAPDH was amplified as a control. The correct sized band (832bp) for δ tryptase was only generated using the F1/R1 primer pair. **B)**. PCR amplification using the purified 832 bp band from Fig. 1A as the DNA template, and the nested primer set NF1/NR1 to generate the expected 698bp product.

Fig. 2. A). The cDNA and putative amino acid sequences of δII tryptase (SEQ ID NO:1). The δII cDNA sequence matched the putative exon sequence of the mMCP-7-like II gene. The δI cDNA (not shown in Fig. 2) matched the exonic sequence of the partial mMCP-7-like I gene. Consistent with the published gene sequences, there were two nucleotide differences between the two cDNA sequences; G^{216} (δII cDNA) to A (δI cDNA) (nucleotide numbering starts from the translation initiation codon), and G^{226} (δII cDNA) to A (δI cDNA). Only the second of these differences results in an amino substitution (Val in δII to Met in δI). Actual nucleotide sequence of cloned RT-PCR product is shown in bold lower case lettering. The location of the forward (NF1) and reverse (NR1) primers are indicated by arrows. The first amino acid of the mature enzyme is italicized and in bold. The three members of the catalytic triad, His, Asp and Ser, are in capitals and underlined. Nucleotide numbering begins from the translation initiation codon (Met). Amino acid numbering begins from the first residue of the mature enzyme (Ile). The position of the forward (●→) and reverse (←●) primers, and the Taqman probe (═) for RTQ-RTPCR are indicated. **B)**. The amino acid sequence of a variant δ tryptase polypeptide. This variant is the product of alternative splicing which results in the excision of 27 nulceotides from the beginning of exon 4, and thus the deletion of 9 amino acids from the polypeptide when compared to the full length δ tryptase polypeptide. The location of the 9 amino acids present in the full length polypeptide but missing in the variant polypeptide is indicated by a vertical arrow (↓).

Fig. 3. Amino acid sequences of δI tryptase and δII tryptase compared to that of tryptases αI, αII, βI, βII and βIII. A dash (-) indicates the presence of an identical amino acid. Numbering begins at the first residue of the mature enzyme, which is indicated by an arrow (▼). The seven loops comprising the substrate binding cleft are boxed and labelled A,B,C,D,1,2,and 3. The H, D and S of the catalytic triad are marked with a hash (#). The premature termination codons of the δ tryptases are marked with an X. The peptide sequence used as the immunogen for anti δ tryptase is underlined (.....).

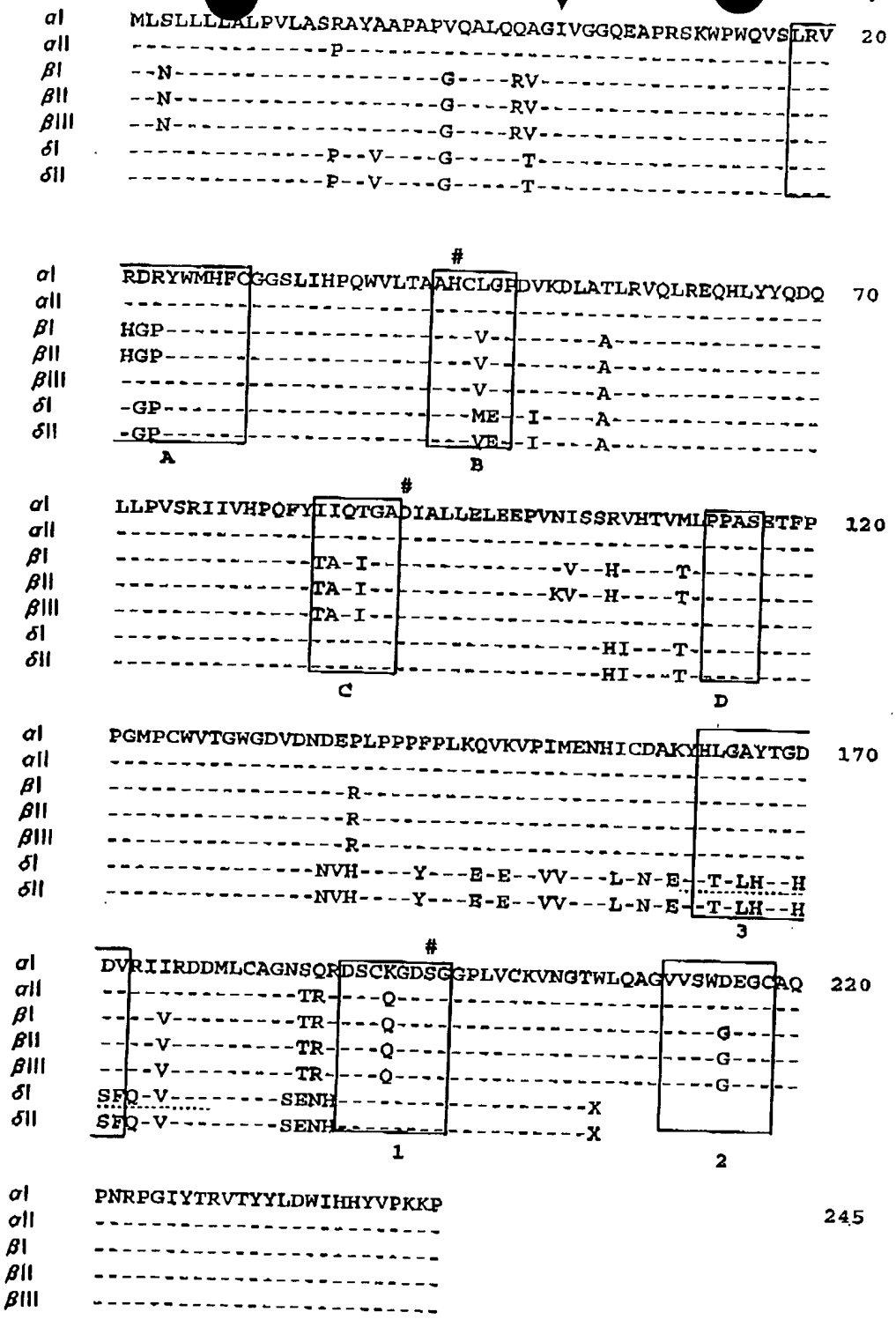

Fig. 3